PHYTOTHERAPY RESEARCH, VOL. **12**, 335–339 (1998)

Antibacterial Constituents from the Rhizomes of *Ferula communis*

Mohammed A. Al-Yahya,* Ilias Muhammad, Humayun H. Mirza and Farouk S. El-Feraly

Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC) and Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia

The rhizomes of *Ferula communis* yielded three antibacterial sesquiterpenes, namely, the new daucane ester 14-(*o*-hydroxycinnamoyloxy)-dauc-4,8-diene (1), ferulenol (2) and ferchromone (3). Compound 1 exhibited significant activity against Gram-positive bacteria, while 3 was found to be less active. Compound 2, on the other hand, demonstrated potent activity against *Mycobacterium* organisms, and its corresponding C-4-acetoxy derivative 9 was found to retain the same activity as well. In addition, the rhizomes yielded a number of inactive compounds, including 2-nor-1,2-*seco*ferulenol, elemicin, colladonin, feselol and compounds 4 and 5. Structural assignments were largely based on the spectral data, especially the 2D NMR COSY and HETCOR experiments. © 1998 John Wiley & Sons, Ltd.

Phytother. Res. **12**, 335–339 (1998)

Keywords: Ferula communis; Umbelliferae; 14-(*o*-hydroxycinnamoyloxy)-dauc-4,8-diene; NMR; antibacterial.

INTRODUCTION

Ferula communis Linn. (Fam: Umbelliferae) is a latex-containing plant that is widely distributed throughout the southern part of Saudi Arabia (Collenette, 1985), where it is known as *Al-kalakh.* The rhizomes of this plant are used locally for the traditional remedy of skin infections, while the roasted flower buds are used against fever and dysentery. Earlier phytochemical investigations on the fruits and the rhizomes have reported the isolation of coumarino-sesquiterpenes (Appendino *et al.,* 1988), daucane esters (Gonzalez and Barrera, 1995; Valle *et al.,* 1987) and phenypropanoids (Miski *et al.,* 1983). A recent literature survey revealed the antimicrobial activity of the essential oil of *F. narthex* (Kar and Jain, 1971) and the gum-resin of *F. gumosa* (Vaziri, 1975), but until now, *F. communis* has not been the subject of antimicrobial evaluation.

Examination of *F. communis* rhizomes, using a bioautography-guided isolation technique, has led to the isolation and characterization of three antibacterial sesquiterpenes, namely, the new daucane ester 14-(*o*-hydroxycinnamoyloxy)-dauc-4,8-diene (**1**), the coumarino-sesquiterpene ferulenol (**2**) and the chromane sesquiterpene ferchromone (**3**). In addition, a number of inactive compounds were also obtained, including the phenylpropionoids elemicin (Muhammad and Waterman, 1985), the two known daucane esters 2α-acetyl-6α-(benzoyl) jaeschkenadiol (**4**) and 2α-acetyl-6α-(p-anisoyl)-jaeschkenadiol (**5**) (Miski and Mabry, 1985), as well as the three known coumarino-sesquiterpenes, 2-nor-1,2-secoferulenol, (Appendino *et al.,* 1989), feselol and colladonin (Miski *et al.,* 1985; Pinar and Rodriguez, 1977). The isolation, structure elucidation and antimicrobial activity of these compounds are the subject of this paper.

* Correspondence to: M. A. Al-Yahya, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC) and Department of Pharmacognosy, College of Pharmacy, King Saud University, PO Box 2457, Riyadh 11451, Saudi Arabia.

MATERIALS AND METHODS

General. Melting points are uncorrected. The NMR spectra were taken on a Varian instrument at 200 or 300 MHz (^{1}H) and 75 or 50 MHz (^{13}C), using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal standard. Multiplicity determination (APT and DEPT/OR DEPTGL) and 2D NMR spectra (COSY and HETCOR) were obtained using standard Varian software. EIMS were run at 70 eV and CIMS were recorded using isobutane as the ionizing gas. Optical rotations were obtained at ambient temperature in $CHCl_3$ (unless otherwise stated) using a Perkin-Elmer 241 MC polarimeter. TLC was performed on silica gel GF_{254} using petroleum ether (BR 40°–60°)-EtOAc (8:2) as solvent, with visualization using vanillin–H_2SO_4 spray reagent. Centrifugal preparative TLC (CPTLC using a Chromatotron® instrument, Harrison Research Inc. Model 7924) was run with 1 mm silica gel PF_{254} disc, using a flow rate of 2 mL/min.

Plant material. The rhizomes of *F. communis* L. were collected in Al-Taif, Saudi Arabia, in April 1990. A voucher specimen was deposited at the herbarium of the MAPPRC, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia.

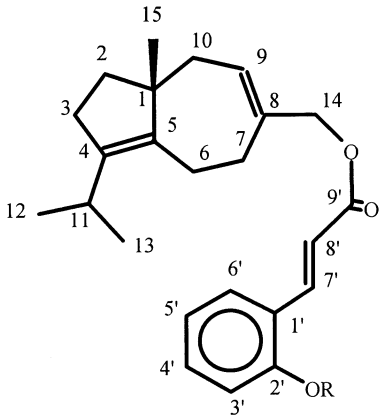
Extraction and bioautography of crude extract. The dried ground rhizomes (800 g) were percolated at room temperature with petroleum ether (40°–60°) and the

CCC 0951–418X/98/050335–05 $17.50

Accepted 5 March 1998

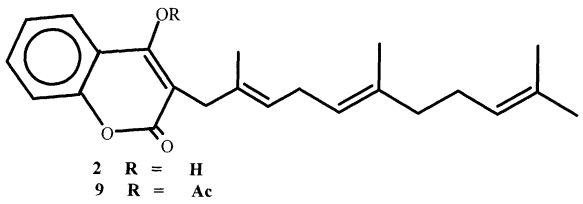
extract was evaporated *in vacuo* to leave 39 g of residue. Preliminary antimicrobial screening showed strong antibacterial activity of the crude petroleum ether extract using agar dilution assay (Mitcher *et al.*, 1972). Hence, the petroleum ether extract was subjected to bioautography (Hamburger and Cordell, 1987) over silica gel TLC plate (5 × 10 cm; solvent: petroleum ether–EtOAc, 7:3), using *B. subtilis* as test organism. Three clear circular zones of inhibition with R_f values between 0.53 and 0.55, 0.45 and 0.47, and 0.38 and 0.40 were observed after 24 h of incubation at 37°C.

Isolation of constituents from rhizomes. The active petroleum ether extract (35 g) was subjected to flash chromatography over silica gel (type 60, 900 g). Elution with petroleum ether (PE, 40°–60°)–EtOAc (1%–6%) yielded 2-nor-1,2-*seco*ferulenol (oil, 60 mg), elemicin (oil, 40 mg) and ferulenol (**2;** needles, 700 mg). Further elution with PE with increasing concentrations of EtOAc (10%–25%) afforded 2α-acetyl-6α-(p-anisoyl)-jaeschkenadiol (**5;** gum, 220 mg), 2α-acetyl-6α-(benzoly) jaeschkenadiol (**4;** plates, 580 mg), compound **1** (gum, 85 mg) and ferchromone (**3;** amorphous powder, 80 mg). Finally elution with 35% EtOAc in PE gave colladonin and feselol (both needles; 50 mg and 80 mg, respectively). All known compounds were identified by comparison of their physical (MP, OR) and spectral data (^{1}H and ^{13}C NMR) with those reported in the literature. Compound **2** (40 mg) was acetylated to **9** using py-Ac_2O following a published procedure (Lamnaouer *et al.*, 1987). Spectral data for **9** were indistinguishable from those reported for 4-acetylferulenol.



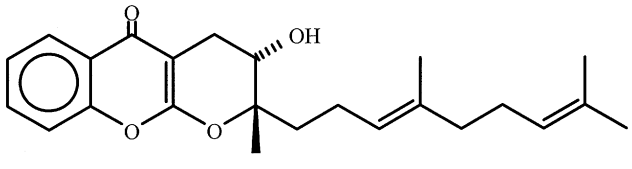
1 R = H

7 R = Ac

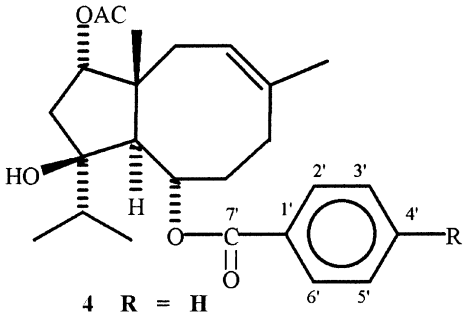


2 R = H

9 R = Ac

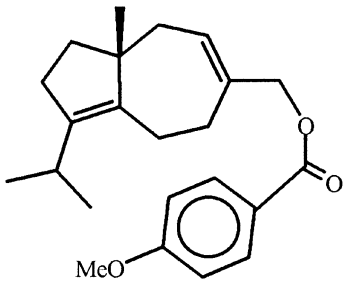


3

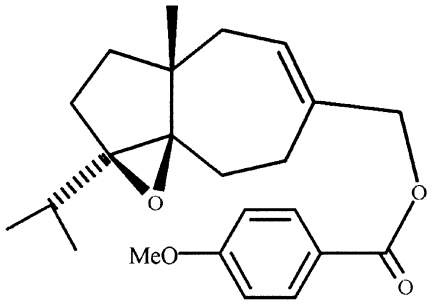


4 R = H

5 R = OMe



6



8

14-*o*-Hydroxycinnamoyloxy)-dauc-4,8-diene (1). Gum; $[\alpha]_D$ +19° (*c* 0.11, $CHCl_3$); UV (λ_{max}, MeOH, nm): 215 (log ε 4.21), 270 (log ε 3.95) and 315 (log ε 3.83); UV (λ_{max}, MeOH + NaOH, nm): 215 (log ε 4.26), 225 (log ε 4.05), 285 (log ε 3.86) and 380 (log ε 2.95); IR (ν_{max} KBr, cm^{-1}): 3500, 2980, 1715, 1605, 1525, 1260 and 760; ^{1}H and ^{13}C NMR: Table 1; EIMS (*m/z*, rel.int.): $[M]^+$ 366 ($[C_{24}H_{30}O_3]^+$, 2), 221 (10), 178 (30), 146 (80) and 118 (100).

Ferulenol (2). Colourless plates from *n*-hexane–EtOAc; MP 70°–72° [lit. (Valle *et al.*, 1987) MP 61°]; UV, IR, ^{1}H and ^{13}C NMR, and MS data were in agreement with those reported for ferulenol (Lamnaouer *et al.*, 1987).

Ferchromone (3). Pale yellow amorphous powder; $[\alpha]_D$ −40° (*c* 0.1, $CHCl_3$); UV, IR, ^{1}H and ^{13}C NMR, and MS data were in agreement with those of ferchromone (Miski and Jakupovic, 1990).

2α-Acetyl-6α-(benzoyl) jaeschkenadiol (4). Colourless needles from *n*-hexane–EtOAc; MP 116°–118° and $[\alpha]_D$ + 12.5° (*c* 0.08 $CHCl_3$) [lit. MP and $[\alpha]_D$ not reported and isolated only as a gum]; UV, IR, ^{1}H NMR and MS data were in agreement with those reported previously (Miski and Mabry, 1985); ^{13}C NMR: Table 2.

Table 1. ^{1}H and ^{13}C NMR chemical shift values for compounds* 1 and 7

C/H	1		7	
	^{13}C	^{1}H	^{13}C	^{1}H
1	49.3 *s*†	-	49.3 *s*	-
2	38.8 *t*	*e*	38.3 *t*	*e*
3	29.8 *t*	*e*	29.4 *t*	*e*
4	139.4 *s*[a]	-	139.4 *s*[a]	-
5	141.3 *s*[b]	-	141.1 *s*[b]	-
6	22.8 *t*	*e*	22.9 *t*	-
7	27.2 *t*	*e*	27.1 *t*	*e*
8	138.1 *s*[a,b]	-	138.1 *s*[a,b]	-
9	128.1 *d*[c]	5.85 br *t* (6.3)	127.5 *d*[c]	5.83 br *t* (6.3)
10	40.3 *t*	2.19, 2.08 2 × *m*	40.3 *t*	2.19, 2.07 2 × *m*
11	26.5 *d*	2.68 *dq* (6.8)	26.5 *d*	2.68 *dq* (6.7)
12	23.7 *q*[d]	0.99 *d* (6.8)[d]	23.6 *q*[d]	0.98 *d* (6.7)[d]
13	21.2 *q*[d]	0.93 *d* (6.8)[d]	20.9 *q*[d]	0.92 *d* (6.7)[d]
14	70.3 *t*	4.63, 4.69 2 × br*d* (12.1)	70.3 *t*	4.62 2 × br*d* (12.0)
15	21.8 *q*	0.93 *s*	21.2 *q*	0.94 *s*
1′	121.7 *s*	-	127.1 *s*	-
2′	155.6 *s*	-	149.2 *s*	-
3′	116.4 *d*	6.87 *dd* (1.0, 8.1)	123.1 *d*	7.12 *dd* (1.0, 8.1)
4′	129.2 *d*[c]	7.24 *dt* (1.5,8.1)	131.4 *d*	7.41 *dt* (1.6,8.1)
5′	118.1 *d*	6.90 *dt* (1.0, 7.5)	126.3 *d*	7.26 *dt* (1.0, 8.1)
6′	131.1 *d*	7.47 *dd* (1.5, 7.5)	128.6 *d*[c]	7.64 *dd* (1.6, 8.1)
7′	141.0 *d*[b]	8.01 *d* (16.3)	137.9 *d*	7.76 *d* (16.1)
8′	120.6 *d*	6.67 *d* (16.3)	120.3 *d*	6.46 *d* (16.1)
9′	168.6 *s*	-	166.0 *s*	-
OAc	-	-	21.8 *q*, 169.0 *s*	2.36 *s*

* All spectra recorded at 300 MHz (^{1}H) and 75 (^{13}C) MHz.
Values in parentheses are coupling constants (*J*) in Hz.
† Multiplicities of the carbon signals were determined by APT and /DEPT experiments.
[a–d] Interchangeable signals [e] Overlapping CH_2-proton signals.

Acetylation of 14-(*o*-hydroxycinnamoyloxy)-dauc-4, 8-diene (1). Compound **1** (40 mg) was dissolved in pyridine (1 mL) and treated with Ac_2O (0.5 mL) at room temperature for 24 h. Regular work-up gave **7** as a transparent gum (35 mg); $[\alpha]_D + 25°$ (*c* 0.05, $CHCl_3$); UV (λ_{max}, MeOH, nm): 215 (log ε 4.25), 278 (log ε 3.98) and 318 (log ε 3.87); IR (ν_{max}, neat): 1760, 1720, 1600, 1530, and 760; ^{1}H and ^{13}C NMR: Table 1.

Evaluation of Antimicrobial activity. The qualitative assay was performed using the agar dilution assay (Mitscher *et al.*, 1972) described in previous communications (Orabi *et al.*, 1991; Muhammad *et al.*, 1992). The bacteria used were National Collection Type Cultures (NCTC) and obtained from Central Health Laboratory, London. They included *Staphylococcus aureus* (No. 6571), *Bacillus subtilis* (No. 10400), *Streptococcus durans* (No. 8307) and *Enterococcus faecalis* (No. 775). *Mycobacterium intracellulare*, *M. xenopei*, *M. chelonei* and *M. smegmatis* (R-7–R-10, respectively), were obtained from the Centers for Disease Control, Atlanta, Georgia, USA. The quantitative assay for determination of minimum inhibitory concentration (MIC) was performed using a two-fold serial dilution assay (Hufford *et al.*, 1975)[1]. All compounds were initially tested using a concentration of 100 μg/mL. After preliminary evaluation to determine the range of MIC values, the concentration of the first tube was decreased to either 40 μg/mL or 30 μg/mL. The MIC, in μg/mL, was recorded as the lowest concentration that prevented visible growth. The anti-infective agents streptomycin sulphate, isonicotinic acid hydrazide and amikacin sulphate were included as positive controls. The solvent, dimethyl sulphoxide (DMSO), was used as the negative control for all experiments.

RESULTS AND DISCUSSION

The petroleum ether (BP 40°–60°) extract of *F. communis* rhizome showed antimicrobial activity using a standard agar dilution assay. Bioautography of the active petroleum ether extract, followed by silica gel chromatography using petroleum ether–EtOAc mixture as eluant (see Material and Methods) resulted in isolation of the three active constituents **1–3** in addition to six inactive compounds (*vide supra*). The antimicrobial sesquiterpene ester **1,** $C_{24}H_{30}O_3$, was obtained as a transparent gum, and was found to contain an acyl group as established by its spectroscopic data (ν_{max} 1715 cm^{-1}, δ_c 168.6), and a phenolic hydroxyl group (ν_{max} 3500 cm^{-1}), confirmed by the bathochromic shift observed in the UV spectrum on addition of NaOH.

The ^{1}H NMR spectral data of **1** (Table 1) was remarkably similar to those of the daucane sesquiterpene ester **6,** but lacked the signals associated with the C-14-*p*-anisoyloxy side chain (Misky and Mabry, 1986). Instead, **1** was concluded to have a *o*-hydroxy-(*E*)-cinnamoyloxy side chain attached to C-14 of a dauc-4,8-diene carbon skeleton (Table 1). On acetylation, **1** afforded the corresponding monoacetate **7** (δ 2.36, 3H, *s;* δ_c 169.0 and 21.8), which exhibited ^{1}H and ^{13}C NMR signals indistinguishable from those of the daucane ring system

Table 2. ^{13}C NMR chemical shift values for compounds* 4 and 5

C	4	5†
1	48.2 *s*‡	48.1 *s*
2	80.7 *d*	80.6 *d*
3	31.2 *t*	31.0 *t*
4	86.5 *s*	86.3 *s*
5	57.6 *d*	57.6 *d*
6	70.3 *d*	69.8 *d*
7	41.2 *t*	41.1 *t*
8	131.5 *s*	131.4 *s*
9	128.1 *d*	128.7 *d*
10	37.5 *t*	37.3 *t*
11	37.3 *d*	37.1 *d*
12	18.5 *q*[a]	18.4 *q*[a]
13	17.4 *q*[a]	17.3 *q*[a]
14	26.3 *q*	26.2 *q*
15	15.6 *q*	15.5 *q*
1′	130.2 *s*	122.5 *s*
2′	129.6 *d*	131.5 *d*
3′	128.5 *d*	113.7 *d*
4′	133.3 *d*	163.8 *s*
5′	128.5 *d*	113.7 *d*
6′	129.6 *d*	131.5 *d*
7′	166.3 *s*	166.0 *s*
OAc	170.7 *s*	170.5 *s*
	21.1 *q*	21.0 *q*
OMe	—	56.5 *q*

* All spectra recorded at 75 MHz.
† Assignments for carbon 3, 9, 10 and 15 of 5 were incorrectly reported by Misky and Mabry (1985). Also, the chemical shift values of H-2, H-6 and H-9 reported by the same authors for both 4 and 5 were not accurate. [a] Interchangeable signals.
‡ Multiplicities of the carbon signals are determined by APT and /DEPT experiments. Assignments were aided by 2D NMR COSY, HETCOR long range HETCOR (for 5) experiments.

of **1.** In addition, the ^{1}H NMR and 2D NMR ^{1}H-^{1}H COSY experiments established the system -CO-$(CH{=}CH)_E$-$C_6H_5(OAc)_O$ for the side chain attached to C-14 of **7,** which permitted the assignment of the signals at δ 7.12, 7.41, 7.26 and 7.64 (for *J*-values, see Table 1) to H-3′–H-6′, respectively (vs δ 6.87, 7.24, 6.91 and 7.47 for H-3′–H-6′ of **1**). Complete ^{13}C NMR data of **1** was assigned by comparison with ^{13}C NMR data of **7** (Table 1) and **8** (Misky and Mabry, 1985).

The minimum inhibitory concentration values, determined by two-fold broth dilution assay, for compound **1** are reported in Table 3. Daucane ester **1** exhibited strong activity against *S. aureus, B. subtilis, S. durans* and *E. faecalis* (MIC values 2.5 μg/mL against all organisms) comparable to those displayed by streptomycin sulphate (MIC values 2.5, 2.5, 1.25 and 1.25, respectively).

The antibacterial sesquiterpenes **2** and **3** were characterized as ferulenol (**2**) and ferchromone (**3**) by comparison of the physical and spectroscopic data [(UV,IR,^{1}H- and ^{13}C NMR, and MS of **2** and **3,** together with acetylferulenol (**9**)] with those reported previously (Valle *et al.,* 1987; Miski and Jakupovic, 1990, respectively).

In addition to its strong antibacterial activity against *S. aureus, B. subtilis, S. durans* and *E. faecalis* (Table 3), ferulenol (**2**) demonstrated potent activity against four *Mycobacterium* strains, using two-fold serial dilution assay. When tested against *M. intracellulare, M. xenopei, M. chelonei* and *M. smegmatis*, compound **2** was found to be more potent (MIC values against each species was 1.25 μg/mL) than its corresponding acetate **9** and ferchromone (**3**) (MIC 5.0 and 50.0 μg/mL against each species), as well as the two positive controls, streptomycin sulphate and isonicotinic acid hydrazide (MIC value 10.0 μg/mL for both compounds against each species). The MIC value of the third positive control, amikacin sulphate, was much lower at 0.25 μg/mL In view of its high level of antimicrobial activity ferulenol is considered a promising potential candidate for further evaluation for activity against pathogenic *Mycobacterium* species.

In the course of isolating the antimicrobial principles from rhizomes, 2-nor-1,2-*seco*ferulenol, elemicin, colladonin, faselol, and compounds **4** and **5** were isolated from bioautography inactive regions. The daucane esters **4** and **5** were identified by comparing their spectroscopic data with those previously reported for 2α-acetyl-6α-(benzoyl) jaeschkenadiol (**4**) and 2α-acetyl-6α-(p-anysoyl) jaeschkenadiol (**5**), respectively. Both compounds were previously isolated from *F. communis* (Misky and Mabry, 1985). Structures **4** and **5** were further substantiated by complete ^{13}C NMR assignments, which have not been previously reported for **4,** using 2D NMR COSY,

Table 3. Minimum inhibitory concentration values in (μg/mL) for diterpenes 1–3 and 9

	MIC value (μg/mL)[a]						
Microorganism	1	2	3	9	Amikac	Strept	Inh
Bacillus subtilis[b]	2.5	0.63	12.5	1.25	0.06	2.5	NT
Staphylococcus aureus[b]	2.5	0.63	12.5	1.25	0.06	2.5	NT
Streptococcus durans[b]	2.5	0.63	12.5	1.25	0.06	1.25	NT
Enterococcus faecalis[b]	2.5	0.63	12.5	1.25	0.125	2.5	NT
Mycobacterium intracellulare[c]	(–)	1.25	50	5.0	0.25	10	10
M. xenopei[c]	NT	1.25	50	5.0	0.25	10	10
M. chelonei[c]	NT	1.25	50	5.0	0.25	10	10
M. smegmatis[c]	NT	1.25	50	5.0	0.25	10	10

[a] MIC values determined by two-fold serial dilution assay.
[b] Incubated for 48 h at 37°C.
[c] Incubated for 72 h at 37°C.
(–), Inactive; NT, not tested; Amika, Amikacin sulphate; Strept, Streptomycin sulphate; Inh, Isonicotinic acid hydrazide.

HETCOR and long-range HETCOR (for **5**) experiments (Table 2).

This appears to be the first report of the daucane ester **1** from a natural source. Furthermore, the *o*-hydroxy-*E*-cinnamoyloxy side chain of **1** had not been previously reported as an ester residue from the genus *Ferula*.

Acknowledgements

The authors thank Dr C. D. Hufford, School of Pharmacy, University of Mississippi, USA, for recording the 300 MHz 1D and 2D NMR spectra, Dr S. Abidin for identification of plant material, Mr Ahmed F. Ramadan and Mr Mustafa O. Khalid for technical assistance.

Notes

[1] The MICs for compounds **1–3** and **9** were also determined by agar dilution assay using Muller-Hinton agar (Oxoid)® as a culture medium and were found to be similar to those recorded by the two fold serial dilution assay. The *Mycobacterium* cultures were grown in Muller-Hinton broth (Oxoid)® at 37°C for 24 h. and after dilution the final inoculum size (10^3–10^4 CFU per zone) was in accordance with those reported by Swenson *et al.* (1982).

REFERENCES

Appendino, G., Tagliapietra, S., Crarotto, G., and Nano, G. M. (1989). Structure and synthesis of a prenylated acetophene from *F. communis. Gazetta Chemica Italiana* **119**, 385–388.

Appendino, G., Tagliapietra, S., Gariboldi, P., Nano, G. M., and Picci, V. (1988). ω-Oxygenated prenylated coumarins from *F. communis. Phytochemistry* **27**, 3619–3624.

Collenette, S. (1985). *An Illustrated Guide to the Flowers of Saudi Arabia* p. 484. Scorpion Publishing Ltd, London.

Gonzalez, A. G., and Barrera, J. B. (1995). Chemistry and source of mono- and bicyclic sesquiterpenes from *Ferula* species. *Nat. Prod. Rep.* **64**, 1–92.

Hamburger, M. O., and Cordell, G. A. (1987). A direct bioautographic TLC assay for compounds possessing antibacterial activity. *J. Nat. Prod.* **50**, 19–22.

Hufford, C. D., Funderbuck, M. J., Morgan, J. M., and Robertson, L. W. (1975). Two antimicrobial alkaloids from heartwood of *L. tulipifera. J. Pharm. Sci.* **4**, 789–792.

Kar, A., and Jain, S. R. (1971). Investigations on the antibacterial activity of some Indian indigenous aromatic plants. *Flavour Ind.* **2**, 111–113.

Lamnaouer, D., Bodo, B., Martin, M.-T., and Molho, D. (1987). Ferulenol and ω-hydroxyferulenol, toxic coumarins from *F. communis* var. *Genuina. Phytochemistry* **26**, 1613–1615.

Miski, M., and Jakupovic, J. (1990). Cyclic farnesyl-coumarin and fernesyl-chromone derivatives from *F. communis* subsp. *communis. Phytochemistry* **29**, 1995–1998.

Miski, M., and Mabry, T. J. (1985). Daucane esters from *F. communis. Phytochemistry* **24**, 1735–1741.

Miski, M., and Mabry, T. J. (1986). New daucane esters from *F. tingitana. J. Nat. Prod.* **49**, 657–660.

Miski, M., Ulubelen, A., Lee, E., and Mabry, T. J. (1985). Sesquiterpene-coumarin ethers of *F. tingitana. J. Nat. Prod.* **48**, 326–327.

Mitcher, L. A., Leu, R.-P., Bathala, M. S., Wu, W.-N., and Beal, J. (1972). Antimicrobial agents from higher plants. 1. Introduction, rationale and methodology, *Lloydia* **35**, 157–166.

Muhammad, I., Mossa, J. S., and El-Feraly, F. S. (1992). Antimicrobial diterpenes from the leaves and seeds of *J. excelsa* M. Bieb. *Phytother. Res.* **6**, 261–264.

Muhammad, I., and Waterman, P. G. (1985). Chemistry in the Annonaceae, XVII. Phenylpropanoids from *Uvariodenran connivens* seeds. *J. Nat. Prod.* **48**, 328–329.

Orabi, K. Y., Mossa, J. S., and El-Feraly, F. S. (1991). Isolation and characterization of two antimicrobial agents from Mace *(M. fragrance). J. Nat. Prod.* **54**, 856–859.

Pinar, M., and Rodriguez, B. (1977). A new coumarin from *F. loscosii* and the correct structure of coladonin. *Phytochemistry* **16**, 1987–1989.

Swenson, J. M., Thornberry, C., and Silcex, V. A. (1982). Rapidly growing *Mycobacteria:* Testing of susceptibility to 34 antimicrobial agents by broth micro dilution. *Antimicrob. Agents Chemother.* **22**, 186–192.

Valle, M. G., Appendino, G., Nano, G. M., and Picci, V. (1987). Prenylated coumarins and sesquiterpenoids from *F. communis. Phytochemistry* **26**, 253–256.

Vaziri, A. (1975). Antimicrobial action of galbanum. *Planta Med.* **28**, 370–373.

